REMARKS

Prior to entering the amendment, Claims 1-6 and 19-24 were pending.

The Amendments

Applicants are re-submitting the entire section of amended specification which was presented before, plus the correction requested in the Office Action.

In view of the claim amendment, Applicants have amended the first paragraph of the specification to delete the priority claim to U.S. Application No. 09/743,103.

At page 3, the description of Figures 1 and 2 were reversed in the original specification. Applicants have corrected the error.

All the trademark names are capitalized with the generic description followed.

At page 26, Lysisbuffer is changed to mtm lysis buffer. Support for the amendment can be found at page 23, lines 14-15.

Based on the Office Action, Applicants understand the Amendment to the Claims dated February 15, 2007 was already entered. Therefore, the current claim amendments are amended from the version of February 15, 2007.

Claim 1 is amended to recite an aqueous lysis buffer comprising SDS. Support for the amendment can be found, for example, at page 8, line 6.

Claim 20 is amended to recite the lysis buffer further comprises one or more detergent. Support for the amendment can be found, for example, at page 8, lines 3-17, and page 15, Table 1.

Support for new Claim 25 can be found, for example, at page 13, line 14.

Support for new Claim 26 can be found, for example, at page 23, lines 13-14.

No new matter is added in any of the above amendments. The Examiner is requested to enter the amendments.

Key Feature of the Amended Claims

To accelerate the allowance of the application, Applicants have amended the claims to recite that the solubilized cervical sample is reacted with an antibody against p16 in the presence

of an aqueous lysis buffer comprising SDS. Although one of the cited references has disclosed lysing cells with a lysis buffer containing SDS, none of the cited references teaches or suggests carrying out the antibody-antigen reaction in the presence of SDS.

SDS is known to be a harsh detergent that causes proteins to denature. For example, SDS is used to denature proteins in size-fractionation by gel electrophoresis. It is well known that SDS in general interferes with antigen-antibody binding reaction because it denatures proteins. **Normally SDS needs to be removed from a sample before an antigen-antibody reaction can be carried out. It is unexpected that the p16 and anti-p16 binding reaction is not interfered by the presence of SDS.**

The key feature of the present invention is not to solubilize p16 from a cervical body sample with a lysis buffer. The key feature is to carry out an immunoassay, which involves the binding of p16 and anti-p16, in the presence of a lysis buffer that normally denatures a protein. The present method is advantageous because it does not require a pre-treatment step to remove the interfering component in a lysis buffer before carrying out an immunoassay for determining the p16 level.

If the Examiner insists that the instant claims are obvious over prior art, **Applicants respectfully request that the Examiner present a reference showing that an immunoassay such as EIA and ELISA can be carried out in the presence of SDS, for measuring p16.**

5. Non-Compliant Amendment

Applicants are re-submitting the corrected version of the Amendment to the Specification dated February 15, 2007.

6. New Matter

Applicants have corrected the description to TWEEN® 20.

9-10. Objection to the Specification

Applicants have amended the specification to capitalize the trademark names with the generic description followed.

Claim 19 is cancelled.

35 USC § 102(e) Rejection

12. Claims 1, 3-6, 23, and 24 are rejected under 35 U.S.C. § 102(e), as allegedly being anticipated by U.S. Patent No. 6,709,832 B1, as evidenced by Geradts et al. (*Am. J. Pathol.* 1999 June; 154 (6): 1665-1671), as evidenced by USPN 6,403,383. The rejection is traversed in parts and overcome in parts in view of the claim amendment.

The '832 Patent does not teach the specific method of the present invention, i.e., reacting the solubilized cervical sample in a lysis buffer comprising SDS with an antibody against cyclin dependent kinase inhibitor p16.

Geradts et al only disclose the Western blot protocol applied on cell monolayers. Geradts does not teach reacting a solubilized cervical sample in a lysis buffer comprising SDS with an antibody against p16. As acknowledged by the Examiner, the Western blot analysis is excluded by the instant claims. Therefore, Geradts is no longer a relevant prior art against the instant claims.

The '383 Patent does not disclose performing an immunoassay by reacting a sample with an antibody in the presence of SDS.

Therefore, the 102(e) rejection of Claims 1 3-6, 23, and 24 should be withdrawn.

Response to Examiner's comments

The '832 Patent discloses different types of body samples including liquor, urine, sputum and blood (column 2, lines 35-38), which are liquid samples and thus no lysis or solubilization is necessary before an immunoassay analysis. The '832 Patent also broadly discloses that the p16 antibodies can be used in Western blot, ELISA, or immunoprecipitation. However, the '832 Patent does not disclose that a solubilized sample can be used directly in ELISA format without any pre-treatment step (separation) step to remove any interfering component in the lysis buffer for an immunoassay. The present invention is a selected invention over the '832 Patent.

35 USC § 103(a) Rejections

13. Claim 2 is rejected under 35 U.S.C. § 103(a), as allegedly being unpatentable over U.S. Patent No. 6,709,832 B1, as evidenced by Geradts et al. (*Am. J. Pathol.* 1999 June: 154 (6): 1665-1671), in view of Ryder et al. (*Clin. Chem.* 1988 Dec; 34 (12): 2513-2516).

Ryder only discloses procedures of enzyme immunoassays using serum as a sample, which does not need to be solubilized or lysed. Ryder does not teach using a sample other than serum or using a detergent in the immunoassays. Ryder does not teach or suggesting performing an immunoassay by reacting a solubilized sample with an antibody in the presence of SDS, thus it does not cure the deficiency of the '832 Patent.

14. Claims 1, 3-6 and 23 are rejected under 35 U.S.C. § 103(a), as allegedly being unpatentable over Khleif et al. (*Proc. Natl. Acad. Sci. USA.* 1996 Apr; **93**: 4350-4354), as evidenced by Bio-Rad Protein Assay (instruction manual provided with a Bradford assay kit manufactured by Bio-Rad) and the American Type Culture Collection™ (ATCC) catalog, in view of Klaes et al. (*Int. J. Cancer.* 2001; 92: 276-284) (of record; cited by Applicant). The rejection is traversed in parts and overcome in parts in view of the claim amendments.

Khleif et al do not teach or suggest reacting a solubilized cervical sample in a lysis buffer comprising SDS with an antibody against p16.

Klaes et al do not teach or suggest solubilizing a cervical body sample in a lysis sample, let alone reacting a solubilized cervical sample in a lysis buffer comprising SDS with an anti-p16 antibody.

Response to the Examiner's comments

Applicants do not agree with the Examiner that contacting a solubilized sample in a lysis buffer with the antibody is an obvious variation of the process that is disclosed by the prior art, particularly in view of the claim amendment. Those skilled in the art would know that immunoassays are sensitive to the interference by a harsh detergent such as SDS, and would either apply a purification step to a solubilized sample to remove the harsh detergent, or to use samples such as urine and serum, which do not need to be solubilized or lysed.

Applicants have discovered that in order to solubilze p16 from a cervical body sample, a harsh lysis buffer containing SDS needs to be used. Such harsh buffer is known to interfere with a typical antigen-antibody reaction. Typically, a solubilized sample needs to be pre-treated to remove SDS or deoxycholate before it can be subjected to an immunoassay. However, Applicants have discovered that the immunoassay of p16 and

anti-p16 can be carried out in the presence of SDS.

The other secondary references, Bio-Rad Protein Assay and ATCC catalog, do not cure the deficiency of Khleif et al. Therefore, the 103(a) rejection of Claims 1, 3-6 and 23 over Khleif et al, as evidenced by Bio-Rad Protein Assay, and ATCC catalog, in view of Klaes et al should be withdrawn.

15. Claim 2 is rejected under 35 U.S.C. § 103(a), as allegedly being unpatentable over Khleif et al. (*Proc. Natl. Acad. Sci. USA.* 1996 Apr; 93: 4350-4354) in view of Klaes et al (*Int. J. Cancer.* 2001; 92: 276-284), and further in view of Ryder et al. (*Clin. Chem.* 1988 Dec; 34 (12): 2513-2516).

Ryder only discloses procedures of enzyme immunoassays. Ryder does not teach or suggesting reacting a solubilized cervical sample in the lysis buffer with an antibody against cyclin dependent kinase inhibitor p16, thus it does not cure the deficiency of Khleif et al or Klaes et al.

Double Patenting Rejection

16. Claims 1, 3-6, and 23 are rejected on the ground of nonstatutory obviousness-type double patenting as allegedly being unpatentable over claims 1, 2, 4, and 5 of U.S. Patent No. 6,709,832 in view of Khleif et al., as evidenced by Bio-Rad Protein Assay.

Claims 1, 2, 4, and 5 of U.S. Patent No. 6,709,832 do not include steps of solubilizing a cervical body sample in a lysis buffer comprising SDS, and reacting the solubilized cervical sample in the lysis buffer with an antibody against cyclin dependent kinase inhibitor p16. The present claims are not an obvious variation of Claims 1, 2, 4, and 5 of U.S. Patent No. 6,709,832 for the same reason as stated above.

Therefore, the double-patenting rejection of Claims 1, 3-6, and 23 over the '832 Patent should be withdrawn.

17. Claim 2 is rejected on the ground of nonstatutory obviousness-type double patenting, as allegedly being unpatentable over U.S. Patent No. 6,709,832 B1 in view of Khleif et al., as evidenced by Bio-Rad Protein Assay, in further view of Ryder et al.

For the same reasons as stated above, Claim 2 is not an obvious variation of Claims 1, 2, 4, and 5 of U.S. Patent No. 6,709,832. Therefore, the double-patenting rejection of Claim 2 the '832 Patent should be withdrawn.

18. Claims 1 and 19-21 are newly rejected under 35 U.S.C. 103(a) as allegedly being unpatentable over US Patent No. 6,709,832, as evidenced by Geradts et al,. in view of U.S. Patent No. 5,889,169, as evidenced by WO 92/20796. Claim 19 is cancelled. The rejection of Claims 1 and 20-21 is overcome in view of the claim amendment.

As discussed above, the '832 Patent and Geradts do not teach or suggest reacting a solubilized cervical sample in a lysis buffer comprising SDS with an antibody against p16.

The '169 Patent and WO 92/20796 also do not teach or suggest reacting a solubilized cervical sample in a lysis buffer comprising SDS with an antibody against p16.

Therefore, the 103(a) rejection of Claims 1 and 20-21 should be withdrawn.

19. Claims 1 and 19-22 are newly rejected under 35 U.S.C. 103(a) as allegedly being unpatentable over US Patent No. 6,709,832, as evidenced by Geradts et al., in view of Ikeda et al. (J. Histochem. Cytochem. 1998; 46:397-403). Claim 19 is cancelled. The rejection of the remaining claims is traversed.

As discussed above, the '832 Patent and Geradts do not teach or suggest reacting a solubilized cervical sample in a lysis buffer comprising SDS with an antibody against p16.

Ikeda et al. disclose a method of protein extraction for Western blot analysis. The tissue sections were lysed in a lysis buffer containing SDS and the lysed samples were used for Western blot analysis. **As acknowledged by the Examiner, Western blot is excluded by the instant claims. Therefore, IKeda et al. do not teach or suggest reacting a solubilized cervical sample in a lysis buffer comprising SDS with an antibody against p16.**

The instant method steps are contrary to a typical antigen/antibody binding assay, which is usually carried out in a mild buffer that does not denature proteins. In a typical immunoassay, SDS is avoided because it is known that SDS denatures proteins in general. Prior to the present invention, it was not expected that an immunoassay relying on the binding of p16 and anti-p16 antibody is not inhibited by the presence of SDS.

Applicants were the first who discovered that p16 could be measured by an anti-p16 antibody in the presence of a lysis buffer comprising SDS. In view of this discovery, Applicants invented a method for detecting the overexpression of p16 by solubilizing a cervical body sample with a lysis buffer comprising SDS and reacting p16 with an anti-p16 antibody in the presence of the lysis buffer. The present method does not need to separate the p16 protein from other proteins in the lysate, or to remove the interfering component in the lysis buffer before carrying out an immunoassay that can determine the level of p16.

Applicants have reduced the invention to practice. Examples 3 and 4 demonstrate solubilizing human cervical swab samples in a lysis buffer comprising SDS, and determining the overexpression of p16 by reacting with an anti-p16 antibody in the lysis buffer. Such method was not taught or suggested by any of the cited prior art, alone or in combination.

Therefore, the 103(a) rejection of Claims 1 and 20-22 should be withdrawn

Request for a Telephone Interview

In order to accelerate the allowance of this application, Applicants have significantly narrowed down the claim scope. In the event that the Examiner still does not find the claims allowable, Applicants respectfully request that the Examiner call the undersigned attorney to resolve the issues and further the prosecution.

CONCLUSION

Applicants believe that the application is now in good and proper condition for allowance. Early notification of allowance is earnestly solicited.

Respectfully submitted,

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